CAN PATHOLOGY SAMPLES DIFFER BETWEEN EE, GERD AND EGID?

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Disclosures
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Consultant and central review pathologist for clinical trials testing therapy for EoE in children:

- GSK
- Cephalon (formerly Ception)
- Meritage

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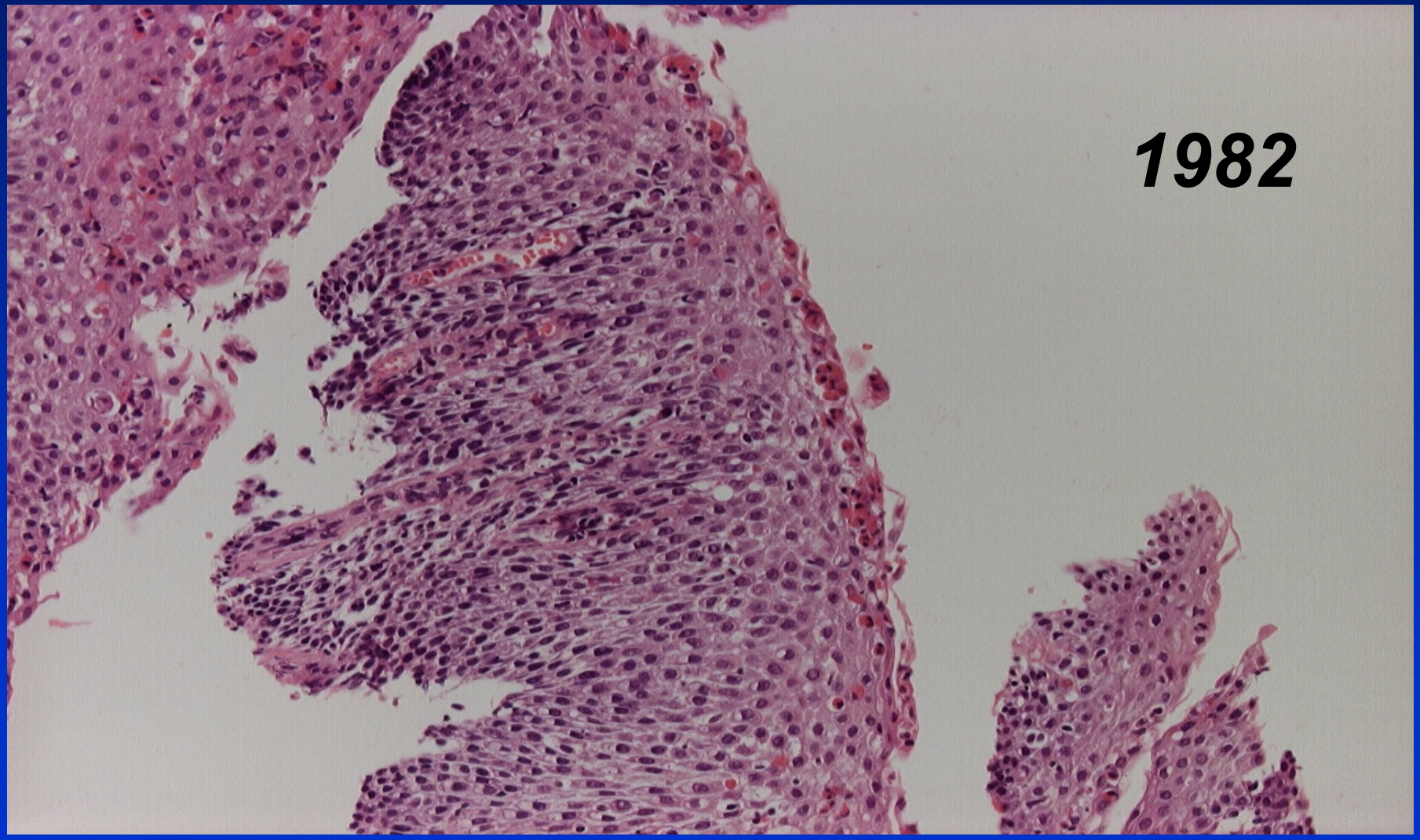
History of histopathology

- Small numbers of eosinophils were reported in esophageal biopsies of patients who had GERD, and eosinophils became pathognomonic of GERD.[1]
- Subsequently, large numbers of intraepithelial eosinophils in esophageal biopsies were reported in adults and children who had normal pH probe tests/did not respond to therapy for GERD, and required/responded to dietary therapy.[2,3]

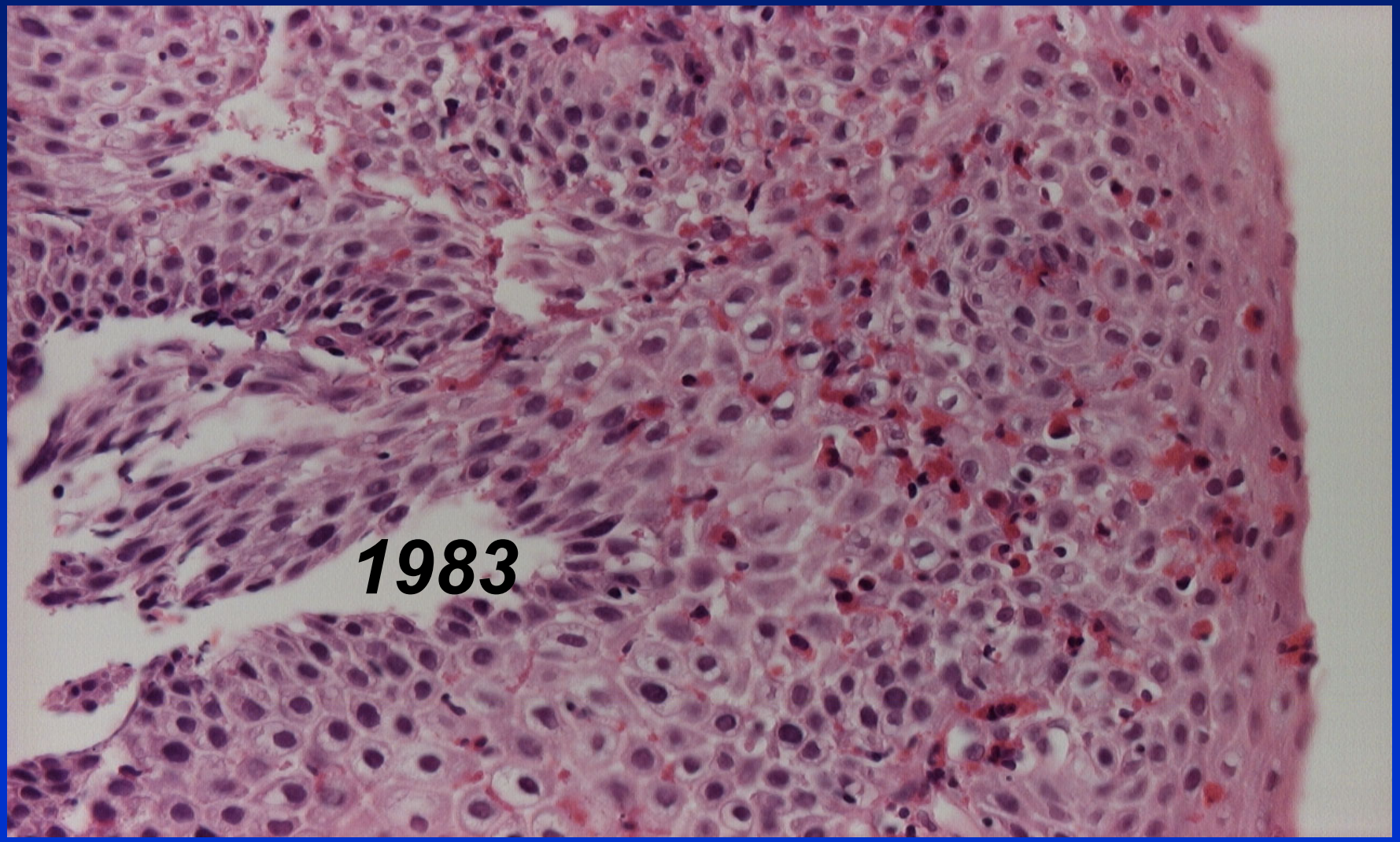
History of histopathology

- More complete descriptions of the histopathology of esophageal eosinophilia followed[4]:
  - Abscesses
  - Surface layering
  - Elongated papillae
  - Basal zone hyperplasia
  - Lamina propria fibrosis[5]

History of histopathology



History of histopathology



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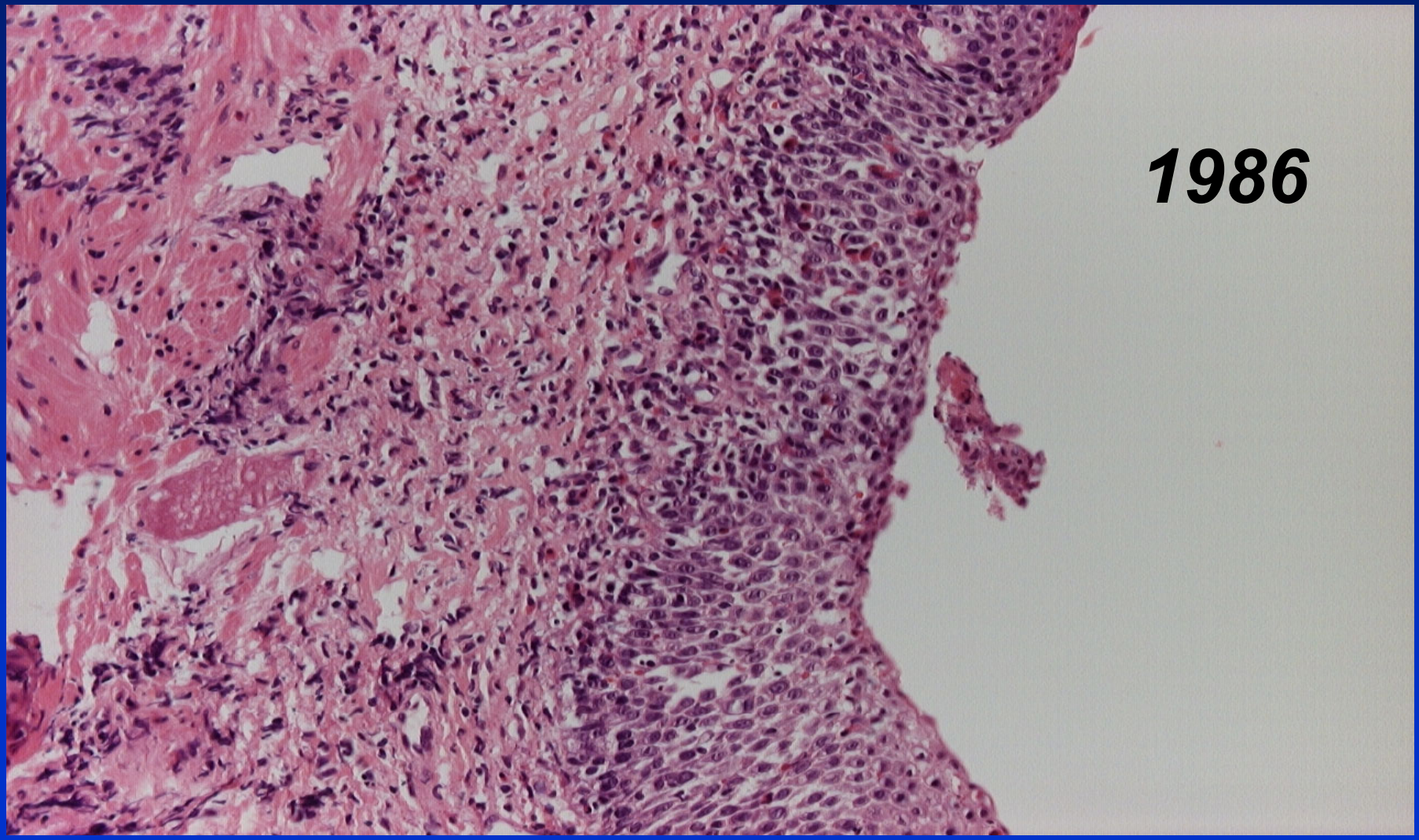
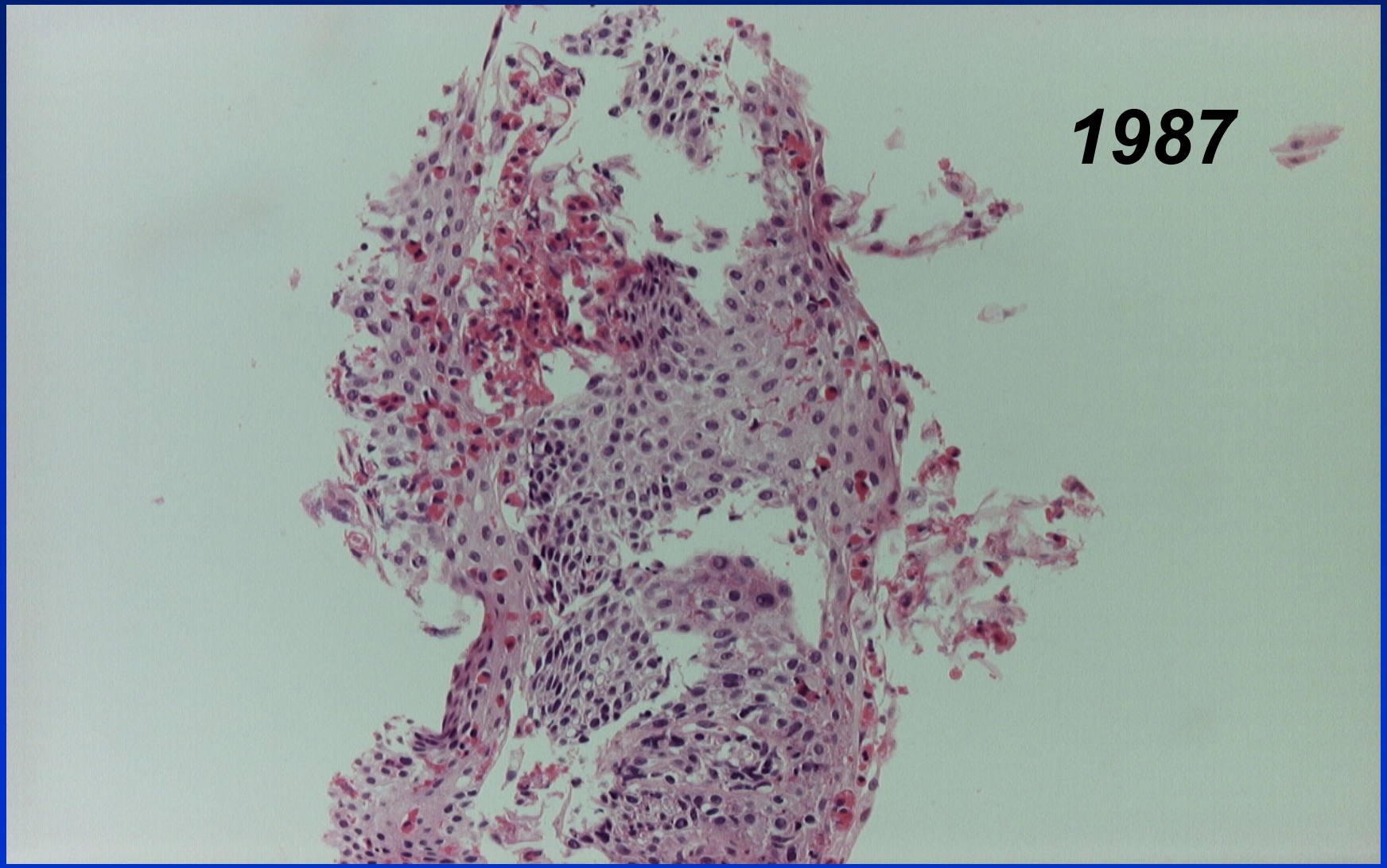
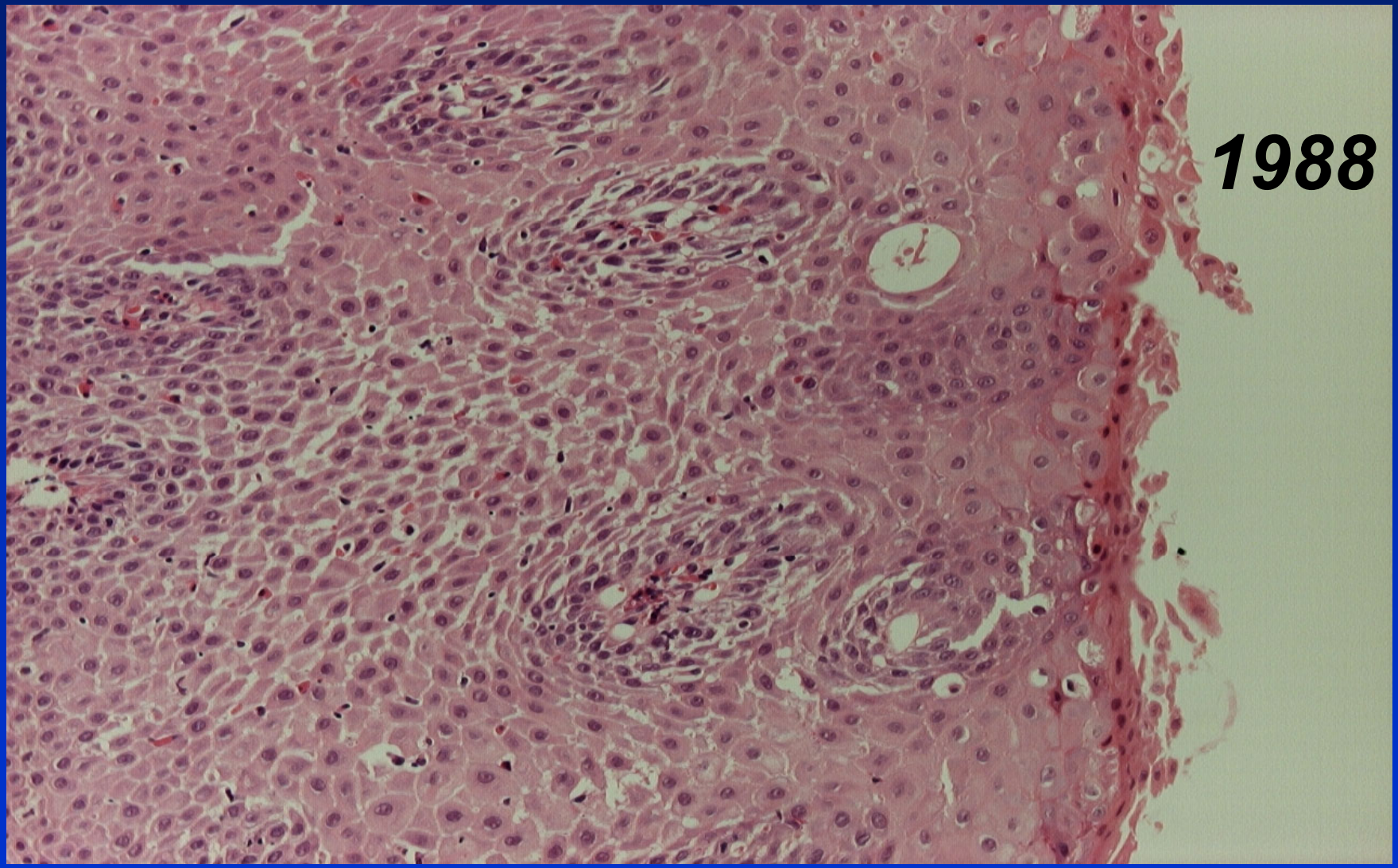


History of histopathology



History of histopathology



History of histopathology

- The histopathology of EoE is not new in either children or adults.[6-10]
- Most studies conclude that the incidence of the histology has remained somewhat stable, but the prevalence has increased due to both increased recognition and increased numbers of endoscopies and biopsies.
- Even small numbers of intraepithelial eosinophils may indicate chronic disease.[6]

How strict are the cut-off for EE?

- Restated: Does a peak eosinophil count ≥15/hpf distinguish EoE from other causes of esophageal epithelial eosinophilia?
- No.
- Again: Do other diseases have ≥15/hpf?
- Yes.
- ***EoE is a clinicopathologic diagnosis. Histopathology must be interpreted in the clinical context.***

EoE vs GERD

MATERIALS AND METHODS

Patients

Between January 1, 1993, and July 1, 1995, 1809 patients were evaluated for GER disease. Each patient displayed chronic gastrointestinal symptoms (>2 months' duration) including abdominal–epigastric pain or regurgitation–vomiting, and at least one of the following: nausea, globus, water brash, chest pain, dysphagia, nighttime coughing, choking, poor appetite, weight loss, or irritability. Upper gastrointestinal series were performed in all patients. Whenever symptoms persisted or recurred despite medical management (positioning, feeding alteration, antacids, or H_2 blockers) or when the symptoms were complicated by gastrointestinal bleeding, respiratory disease, or weight loss, an esophagogastroduodenoscopy (EGD) was performed by a board-certified pediatric gastroenterologist using an Olympus video endoscope (Olympus, Columbia, MD, U.S.A.) (N30, XP20, or GIF 100—depending on the patient's age). Patients with known gastrointestinal disorders (Crohn's disease, ulcerative colitis, celiac disease), anatomic abnormalities (malrotation, hiatal hernia, duplication) or systemic disease (cancer, chronic renal disease, scleroderma) were excluded.

Five hundred eighty-three patients underwent EGD for GER. Of this group, reflux esophagitis was diagnosed and treated in 418. Initial treatment consisted of ranitidine with metoclopramide or cisapride. Those patients who improved during the therapy were considered to be the reference group of children with GER. If the patients' symptoms worsened or did not show at least a 50% improvement with medication, the dose of ranitidine was increased to a maximum of 3 mg/kg twice daily, and patients were given cisapride at a maximum dose of 0.3 mg/kg four times daily. After 3 months, another EGD was performed if symptoms remained despite the therapy. Patients with continued histologic evidence of esophagitis continued receiving cisapride but with the addition of omeprazole (1 mg/kg per day; minimum, 10 mg/day; maximum, 20 mg twice daily).

Patients who remained symptomatic despite more than 3 months of omeprazole and cisapride therapy again underwent EGD (11.2 ± 3.8 months after the initial EGD). Those in whom continued severe esophageal eosinophilia was demonstrated, consisting of more than 15 eosinophils/HPF without evidence of antral or duodenal eosinophilia were defined as the study population. In patients with antral or duodenal eosinophilia, in addition to esophageal eosinophilia, eosinophilic gastroenteritis was diagnosed, and the patients were excluded from the study. Figure 1 outlines selection of the study population.

Pathologic Evaluation

At least two random grasp biopsies were taken of the duodenum (third portion), stomach (antrum), and esophagus (3–5 cm above Z-line) in every patient. Additional biopsies were taken from any other site that was visually abnormal. The average number of biopsy specimens taken from each site was 2.3 ± 1.1. Each specimen was evaluated for inflammatory infiltrate (neutrophils, eosinophils) and ulceration. In addition, duodenal specimens were evaluated for villous and crypt abnormalities, antral specimens for the presence of *Helicobacter pylori*, and esophageal specimens for reflux esophagitis (8,9). In every esophageal specimen, the number of eosinophils per HPF (magnification, ×40) was determined by averaging the number of eosinophils in sequential HPFs over the entire area (one level) of the specimen.

Study Population

All study patients underwent 24-hour pH probe testing (to determine whether acid reflux was related to esophageal eosinophilia) and laboratory evaluation, including complete blood count with differential, total serum eosinophil count, quantitative immunoglobulin (Ig) E level, chemistry panel, and sedimentation rate. Patients with severe reflux revealed by pH probe (reflux index >40%) underwent surgical evaluation for fundoplication. The remaining patients began a 4-week course of oral methylprednisolone. Before therapy, each patient and family member was interviewed and completed a questionnaire regarding the type, chronicity, and frequency of symptoms (including emesis, regurgitation, heartburn, globus, dysphagia, ir-

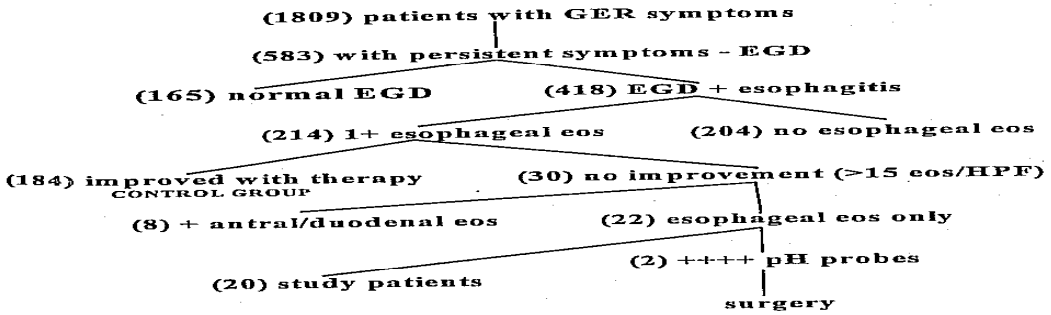


FIG. 1. Flow chart depicting the selection of study patients.

EoE vs GERD

- Early studies identified patients with ≥15 eosinophils/hpf who did not respond to anti-reflux therapy and who had abnormal pH probe studies.[11]
- Some patients who respond to anti-reflux therapy, and may have abnormal pH probe studies, have EoE histology.[12-15]

EoE vs GERD

- 712 adults with upper GI symptoms[16]
- 35/712 (4.9%) had ≥15/hpf pretherapy in biopsies from upper/mid esophagus
- Treated with PPI; response = resolution of clinical symptoms with <5 eosinophils/hpf on repeat biopsy
- 26/35 (74%) responded to PPI
- 9/35 (26%) did not = EoE

Histopathologic findings, n (%)	PPPI-R 15-35/hpf N=17	PPI-R >35/hpf N=9	EoE N=9
Superficial distribution	5 (29%)	6 (66%)	5 (55%)
Degranulating eosinophils	12 (70%)	7 (77%)	7 (77%)
Eosinophils microabscesses	2 (11%)	3 (33%)	5 (55%)
Basal cell hyperplasia	12 (70%)	9 (100%)	8 (88%)
Papillae elongation	12 (70%)	7 (77%)	7 /77%)
Intercellular edema	15 (88%)	8 (88%)	8 (88%)
Lamina propria present	9 (52%)	4 (44%)	7 (77%)
Lamina propia fibrosis	6 (35%)	4 (44%)	7 (77%)
➢ 5 eo in lamina propia	3 (17%)	3 (33%)	6 (66%)
pH monitoring Normal/Pathologic	0/7	2/5	4/3

PATHOLOGY OF EoE

- Eosinophil-predominant inflammation, often with many more than 15 eosinophils/hpf, associated with abscesses, surface layering, epithelial hyperplasia, etc is highly characteristic but ***not pathognomonic*** of EoE.
- The distribution of the changes in the esophagus are also ***not pathognomonic*** of EoE.

Are there other criteria to use in questionable cases?

- Degranulation, microchips, gene expression?
  - Yes, these can be helpful, but require stains in addition to the usual H&E and are best considered research tools currently.

Are there other criteria to use in questionable cases?

- Extracellular eosinophil granules increase as the number of eosinophils increase, and mechanical factors may induce deposition.[17,18]
- Extracellular deposits of eosinophil granule contents may be extensive even in biopsies with few intact eosinophils, and may identify EoE in questionable cases.[15,19-21]
- The number of mast cells is increased in EoE compared to GERD.[15,22]

Are there other criteria to use in questionable cases?

- IgE-positive cells are found in EoE biopsies but few if any are present in GERD.[22-25]
- Genome-wide association studies have identified numerous genes upregulated in EoE, with confirmed increased mRNA and protein expression, including eotaxin-3, periostin and TSLP.[26-30]

Are there other criteria to use in questionable cases?

- Down-regulated genes include filaggrin and involucrin that are essential for epithelial barrier integrity.[31]
- IL-5 gene expression (determined by mRNA) is detected only in EoE.[30]

How do you distinguish the person who comes in already on empiric therapy?

- Biopsies from patients on empiric therapy can be problematic.
- Biopsies from patients on anti-reflux therapy only that show the characteristic histopathology of EoE fulfill the criteria for a diagnosis of EoE.
- Biopsies from patients on anti-reflux therapy and EoE therapy that show the histopathology of EoE fulfill the criteria for diagnosis of EoE.

How do you distinguish the person who comes in already on empiric therapy?

- Biopsies from patients on anti-reflux therapy and therapy for EoE that do not show eosinophil-predominant inflammation could represent either disease. Repeat biopsy after discontinuing EoE therapy may be required.

How to make the diagnosis of EG/EC?

- Eosinophils normally exist in colon and stomach.
- Eosinophils normally exist everywhere in the GI tract except the esophagus.
- Early descriptions of eosinophilic GI disease were mostly based on bowel resections that emphasized changes in the wall.

How to make the diagnosis of EG/EC?

- Few studies have attempted to establish norms for eosinophil concentrations in mucosa throughout the GI tract.[18,32,33]
- Histologic criteria for diagnosis of eosinophilic disorders in sites other than the esophagus do not exist.
- Nonhistologic markers, such as abnormalities of gene structure or expression, do not exist for eosinophilic diseases other then EoE.

How to make the diagnosis of EG/EC?

- In my own practice, numerous eosinophils without signs of epithelial invasion or chronic changes is referred to as mucosal eosinophilia, and biopsies that show eosinophil-predominant inflammation associated with infiltration and damage are diagnosed as eosinophilic gastritis or enteritis or colitis. The presence of any acute inflammatory cells raises the possibility of idiopathic inflammatory bowel disease.

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